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New Animal Models for Parkinson's Disease

Minireview

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Parkinson's disease (PD) is the second most common progressive neurodegenerative disorder primarily affecting individuals between the ages of 50 and 60, although young adults and even children can be affected by this devastating disease (Lang and Lozano, 1998). In the last few years, significant progress has been made into unraveling the causes of PD. In particular, genes linked to familial PD have been identified yielding fresh avenues of investigation. In other neurodegenerative disorders animal models of human familial-linked disease have provided tremendous insight and clues into the pathogenesis of these disorders. Until recently, there has not been an animal model of hereditary PD. Two recent papers by Masliah and colleagues (Masliah et al., 2000) and Feany and Bender (2000) describe such models and provide us with the opportunity to discuss recent advances in our understanding of PD pathogenesis.

Etiology of Dopamine Neuronal Dysfunction and Death

PD is due to a relatively selective degeneration of dopamine (DA) containing neurons in the midbrain. The loss of DA neurons is accompanied by the characteristic formation of Lewy bodies (cytoplasmic accumulations of aggregated proteins) in cell bodies and neurites. Lewy bodies and neurites are mainly found in DA neurons, but they can be identified in other neuronal systems. Most Lewy bodies stain abundantly with ubiquitin, a key player in protein degradation. PD patients experience slowness of movement, rigidity, tremor, and difficulty with balance. Unlike for most other neurodegenerative disorders there is effective temporary symptomatic treatments for PD involving DA replacement and other adjunctive medications or surgical approaches (Lang and Lozano, 1998). Because the neurodegeneration in PD is progressive and there is no proven preventative, restorative, or regenerative therapy, patients ultimately become quite disabled.

The majority of PD is sporadic, although there are rare genetic familial forms of the disease (see below). Insight into the cause of sporadic PD has come from postmortem examination of affected brains. In addition to loss of DA neurons and the presence of Lewy bodies, there are indications of increased oxidative stress such as glutathione depletion, iron deposition, increased markers of lipid peroxidation, oxidative DNA damage, and protein oxidation (Dunnett and Bjorklund, 1999). Another key postmortem finding is decreased expression and activity of mitochondrial complex 1 in the midbrain. This defect is specific, as it does not occur in other brain areas, and it has not been observed in other neurodegenerative diseases. Other components of the mitochondrial respiratory chain are unaffected in PD. Thus, in sporadic PD, oxidative stress and mitochondrial dysfunction appear to play prominent roles in the death of DA neurons, perhaps through a combination of excitotoxic, necrotic, and apoptotic mechanisms (see Dunnett and Bjorklund, 1999 and references therein).

Identification of Familial-Linked PD Genes Yield New Insight into PD Pathogenesis

The discoveries of genetic linkages for PD provide promise for new insights into the pathogenesis of the disease. Rare missense mutations in α-synuclein (α-syn), A53T, and A30P cause autosomal dominant PD in a large Italian, American, Greek kindred and a small German pedigree, respectively (Polymeropoulos, 1998). α-syn and related family members are abundant neuronal cytosolic proteins enriched at presynaptic terminals and are thought to be involved in synaptic function and plasticity (Clayton and George, 1998). A truncated fragment of α-syn was also identified as the non-β-amyloid component of Alzheimer's disease senile plaques (Ueda et al., 1993). Following the discovery of the α-syn mutations, α-syn was quickly discovered to be a major component of Lewy bodies and neurites, and it is abundant in pale bodies, which are believed to be the precursor of Lewy bodies (Trojanowski et al., 1998). Abnormal deposition of α-syn also occurs in a variety of neurodegenerative disorders, many of which have parkinsonian features (Trojanowski et al., 1998). Wild-type (WT) and mutant (A53T and A30P) α-syn self-aggregate and assemble into fibrils that resemble the ultrastructural elements of Lewy bodies (see Conway et al., 2000 and references therein). The altered conformation of both α-syn mutants may somehow contribute to the demise of DA neurons. It is tempting to speculate that the reported decreases in mitochondrial complex 1 activity and increased oxidative stress in sporadic PD could lead to an oxidative environment that predisposes WT α-syn to obtain an altered toxic conformation or promote α-syn fibril formation (Figure 1). Recent in vitro and in vivo data indicates that oxidative stress can induce α-syn aggregation (see Kowall et al., 2000 and references therein). Whether the toxic moiety of α-syn is the altered conformation or whether it is the assembly into fibrils that kills DA neurons is not known. However, the recent observation that the A30P α-syn mutant fibrillizes more slowly than WT or A53T α-syn suggests that fibrils may not be the toxic moiety (Conway et al., 2000). On the other hand, both mutants oligomerize more rapidly than WT α-syn potentially implicating the process of oligomerization and protofibrils in the pathogenesis. It is possible that fibrils and Lewy bodies are an attempt at detoxification of mutant or damaged α-syn (Conway et al., 2000); pathologic studies have suggested that Lewy bodies are neuroprotective (Forno, 1996). In a similar manner, it has been proposed that nuclear inclusions of polyglutamines in trinucleotide repeat disorders, such as Huntington's disease and the spinocerebellar ataxias, may serve a protective role (Orr and Zoghbi, 2000).

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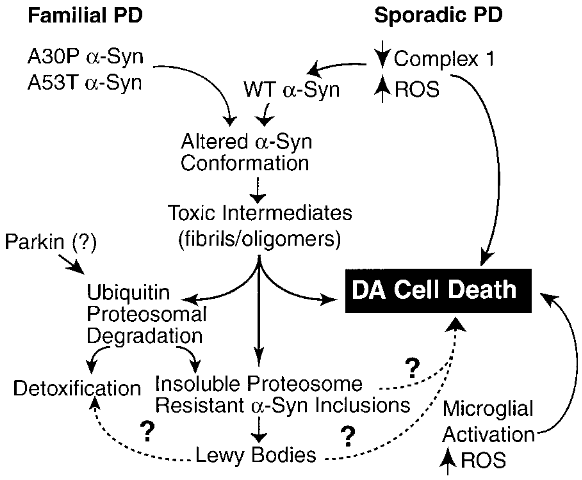

Figure 1. α-Synuclein-Centric Model of DA Cell Death in PD

α-syn, either through genetic mutation or via oxidative damage through decrements in mitochondrial complex 1 and reactive oxygen species (ROS), obtains an altered conformation that promotes the formation of toxic intermediates and α-syn inclusions. In this model Parkin participates in the detoxification of α-syn by participating in ubiquitin-mediated proteosomal degradation. α-syn could be a target for ubiquitination at the toxic intermediate stage or at insoluble inclusion stage (not shown). Processes that interfere with the ubiquitin proteosomal degradation pathway or the formation of α-syn inclusions could accelerate the death of DA neurons. (See text for full discussion of model.)

α-syn also binds to a variety of proteins that could ultimately contribute to the toxic properties of α-syn as some of these protein interactions are potentiated by the α-syn mutations. A potentially important interactor is synphilin-1. Cotransfection of a truncated form of α-syn, the nonamyloid component of amyloid plaques (Ueda et al., 1993), with synphilin-1 leads to the formation of cytoplasmic inclusions (Engelender et al., 1999). Furthermore, synphilin-1 is concentrated in Lewy bodies in PD and diffuse Lewy body disease (Wakabayashi et al., 2000). It is conceivable that mutant or damaged α-syn interacts with synphilin-1 to create the toxic intermediate. Why would perturbations in α-syn lead to selective DA neuron death? The propensity of DA cells to degenerate with abnormalities in α-syn may be related to the unique role of α-syn in DA physiology as mice lacking the gene for α-syn display selective alterations in DA release, reductions in striatal DA, and attenuation of DA-dependent locomotor response to amphetamine (Abeliovich et al., 2000).

Other genes have been linked to familial PD. Mutations in *parkin* cause autosomal recessive PD (Mizuno et al., 1999). Patients with mutations in *parkin* develop many of the features of sporadic PD, but tend to manifest disease at an earlier age including childhood. Most of the mutations identified are thought to inactivate the gene through various homozygous deletions in exons 3 to 7, but recent investigations suggest that missense mutations in *parkin* can also cause PD that appears indistinguishable from sporadic PD (Abbas et al., 1999). Parkin is a protein of unknown function, but sequence homology to ubiquitin in its N terminus suggests that it may function in the ubiquitin protein degradation pathway. The C terminus contains two ring finger motifs and an IBR (in between ring finger) domain. The ring finger and IBR domains could function as a protein interaction and/or a transcriptional activator domains. Patients with mutations in *parkin* show a severe loss of DA neurons and the absence of Lewy bodies (Mizuno et al., 1999). In contrast, Parkin immunoreactivity is present in Lewy bodies of sporadic PD. The mechanism by which Lewy body–associated proteins are targeted for degradation has not been identified. Although speculative in nature, the absence of Lewy bodies in patients with mutations in *parkin* would fit with a hypothesis that Parkin might be involved in the ubiquitin-mediated degradation of Lewy body–associated proteins. Consistent with the notion that Parkin might function in the ubiquitin pathway is the observation that a number of unrelated proteins with similar ring finger and IBR domains serve as substrates for ubiquitination and may function as ubiquitin-conjugating enzymes (Lorick et al., 1999). If Parkin is critical in this process, then in the absence of Parkin, damaged α-syn would not be ubiquitinated and proteosomal degradation would not occur. Lewy bodies would not form, damaged α-syn would be free to kill DA neurons, and disease would be more severe and occur earlier (Figure 1). In this model α-syn could still form inclusions, but they would not be ubiquitinated. It will be of interest to determine whether there are α-syn-positive inclusions in patients with mutations of *parkin*. In a similar manner, disruption of ubiquitin-mediated degradation of polyglutamine repeat expansions in the trinucleotide repeat disorders significantly retards the formation of inclusions and accelerates disease (Orr and Zoghbi, 2000).

Autosomal dominant familial PD has also been linked to ubiquitin carboxy-terminal hydrolase L1 on chromosome 4P. Other as yet unidentified genes have been linked to chromosomes 2P and a different site on 4P (see Mizuno et al., 1999 and references therein). Families with hereditary PD have also been described, but linkages have not yet been associated with any known genes or chromosomes. Identification of these genes will be important in our quest to understand the pathogenesis of PD, as understanding the normal physiological and pathological actions of these proteins will contribute insight into the pathogenesis of this disease.

Experimental and Animal Models of PD

Insight into the molecular mechanisms of DA cell death has come from the study of the 6-hydroxydopamine (6-OHDA) and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) models of PD (Dunnett and Bjorklund, 1999). Attention has been directed toward the MPTP model as it more faithfully recapitulates many of the features of sporadic PD. The accidental use of MPTP by heroin addicts led to the discovery that this toxin causes PD (see Lang and Lozano, 1998 and references therein). In humans, nonhuman primates, and rodents, MPTP elicits many of the biochemical, neuropathological, and clinical features of PD. The active metabolite of MPTP, 1-methyl-4-phenyl pyridium (MPP^+), elicits selective DA neuronal degeneration by concentrating in DA neurons via the high-affinity DA transporter. MPP^+ is a selective and potent mitochondrial complex 1 inhibitor, which led to the discovery of reduced mitochondrial complex 1 activity in sporadic PD. If it were not for the acute nature of MPTP intoxication, it would be an ideal model for sporadic PD. Most of the current theories on the mechanisms of cell death in PD stem from studies of the MPTP model, which has yielded many important insights (Dunnett and Bjorklund, 1999). MPTP appears to elicit DA degeneration through inhibition of mitochondrial complex 1 and the formation of the superoxide anion. This coupled with generation of both neuronal- and microglial-derived nitric oxide to form peroxynitrite, which oxidatively injures DA neurons, perhaps through DNA damage and activation of poly (ADP-ribose) polymerase may be major contributors to DA neuron death (Grunewald and Beal, 1999). In addition, there is emerging evidence for neuroinflammation in the pathogenesis of MPTP-induced parkinsonism (Liberatore et al., 1999). The inflammatory component is an attractive target for therapeutic intervention. Interestingly, MPTP administration in nonhuman primates induces α-syn aggregation in degenerating DA neurons, thus linking decrements in mitochondrial complex 1, oxidative stress and α-syn aggregation (Kowall et al., 2000) (Figure 1).

The discovery of genes that are linked to familial PD provides us with the opportunity to generate animal models that may more faithfully recapitulate both the phenotypic and pathologic features of PD, particularly with regards to the age-dependent onset and the progressive nature of a degenerative illness. Masliah and colleagues (Masliah et al., 2000) developed a mouse model of WT human α-syn overexpression using the platelet-derived growth factor-β (PDGF-β) promoter. They characterized five lines of mice that express normal levels of mouse α-syn plus 10%–80% of α-syn levels found in human brain. All of these transgenic strains developed neuronal cytoplasmic and nuclear inclusions that stained with antibodies to human α-syn and ubiquitin. Nuclear inclusions are atypical in sporadic PD. The inclusions seem to get larger with age, which may be consistent with a degenerative process. Electron microscopy revealed electron-dense inclusions composed of fine granular material, although no fibrillar aggregation, which is characteristic of Lewy bodies, was noted. There was no reported loss of DA neurons, however, in the 12-month-old high-expressing line there was a significant degeneration of DA nerve terminals. There were also decrements in the levels and catalytic activity of the rate-limiting enzyme of DA biosynthesis, tyrosine hydroxylase (TH), with an accompanying impairment in rotorod motor performance. It is difficult to say whether the motor deficits are due to the small decrements in TH activity, as symptoms in PD patients and in the MPTP models develop after losing a critical threshold of 60% to 80% of striatal DA (Dunnett and Bjorklund, 1999). This should be readily addressable in future behavioral studies monitoring the response to DA replacement.

Feany and Bender (2000) have developed a fruit fly model of PD associated with overexpression of α-syn. When normal and mutant forms of α-syn are overexpressed in *Drosophila*, the flies develop an adult-onset (mid-life) progressive loss of DA neurons and filamentous intraneuronal inclusions that contain α-syn. The transgenic flies also develop progressive age-dependent locomotor dysfunction. It is not yet known, whether the motor deficits of transgenic flies respond to DA replacement, but this should also be easily testable in future studies as there are reliable ways to administer and monitor motoric responses of fruit flies to DA agents. A particularly striking observation in the transgenic α-syn fruit flies is the selective loss of DA neurons. When α-syn is expressed in a panneural distribution using the *elav*-GAL4 driver, the flies developed a marked age-dependent loss of dorsomedial DA neurons. Interestingly, not all DA neurons degenerate in α-syn transgenic flies, as a number of non-dorsomedial TH-positive cells remain in aged flies that express α-syn in a panneural distribution or selectively in DA neurons. This is reminiscent of the sparing of subsets of DA neurons in patients with sporadic PD. The flies develop α-syn-positive inclusions and electron microscopy reveals intracytoplasmic inclusions that have a relatively homogenous core and are edged by radiating filaments projecting into a surrounding halo. The inclusion formation parallels α-syn toxicity both in its restriction to the nervous system and its timing. The overall appearance of the inclusions is highly similar to human Lewy bodies. The authors did not show whether these inclusions stain for ubiquitin (Lewy bodies are typically enriched in ubiquitin), but the presence of high background ubiquitin staining in the *Drosophila* nervous system may prevent a definitive identification. When α-syn is expressed specifically in the developing and adult eye, retinal degeneration occurs in an age-dependent manner and illustrates that degenerative change can occur in nondopaminergic cells similar to neuronal degeneration of non-DA systems in human PD-related syndromes.

How Do These New Models Compare to Existing Models?

Although work still needs to be done to fully characterize both models, the major advantage over existing models is their chronic nature and the presence of α-syn inclusions. The fact that expression of WT human α-syn elicits DA deficits, neuronal inclusions, and motor deficits in species as diverse as fruit flies and mice makes it likely that the alterations induced in these animal models are also involved in the human disease. The fly model fulfills most of the criteria for an excellent PD model, including progression, age dependence, selective loss of DA neurons, and formation of fibrillar α-syn inclusions. Although the present mouse model represents a significant advance, the fly model is quite compelling and suggests that we should be able to develop a mouse that recapitulates sporadic PD more faithfully than the present model. Perhaps aging the α-syn transgenic mice beyond 1 year or increasing the level of neuronal oxidative stress through pharmacological or genetic means will produce more significant pathology and DA cell loss. If we extrapolate the findings from *Drosophila*, we might need to examine mice that are significantly older than one year. Alternatively, expressing higher levels of human WT α-syn or the mutant α-syn should produce transgenic mice with a more severe phenotype.

Do These Transgenic Models Teach Us Anything New about PD Pathogenesis?

These models are quite persuasive in implicating α-syn in the pathogenesis of PD. Due to the timing of α-syn inclusion formation in the fly, the process and/or formation of α-syn inclusions appear to be the cause of DA neuron cell death. The mouse model also teaches us that α-syn accumulation without the formation of fibrils leads to DA neuron dysfunction. α-syn inclusions are not restricted to DA neurons and yet none of these other neuronal subtypes show obvious degeneration. As yet, neither model yields any specific clues on how α-syn selectively injures or kills DA neurons. In particular, questions regarding the role of α-syn fibrils, protofibrils, oligomers, pale bodies, Lewy bodies, ubiquitination, and α-syn-interacting proteins in DA neuron cell death remain. Furthermore, the relationship and timing of oxidative stress and decrements in mitochondrial complex 1 to α-syn aggregation in sporadic PD remain to be shown. Whether microglia participate in α-syn-mediated DA neuron death is not known. However, the fly and mouse models should prove enormously useful in clarifying these complex issues. Suppressor and modifier screens in the fly could also identify important pathways involved in α-syn toxicity, which could be exploited for novel therapeutics in the treatment of PD.

Selected Readings

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